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SHORT-TERM PRESERVATION OF *MOINA MICRURA*: INFLUENCE OF STORAGE ON PROTEIN, LIPID and CARBOHYDRATE CONTENTS

Srivastava P.K.
Department of Zoology, Devendra Post Graduate College, Bilthra Road, Ballia, Uttar Pradesh. (India). Contact Number: +91-9919441980, Email: srivp@yahoo.com.

ABSTRACT
Continuous supply of fresh zooplankton for the fish is not assured throughout the year as it depends largely on weather. Therefore, preservation of zooplankton is necessary, but freezing and preservation process results in deterioration of its nutritional values. In the present study, preservation of *Moina micrura* at three different temperatures, +4 ºC, –4 ºC and –20 ºC resulted in devaluation of protein, lipid and carbohydrate contents that progressively increased with progress of time. At +4°C, the loss in protein, lipid and carbohydrate contents was 30.16%, 55.32% and 37.27% after 7 day of preservation respectively. The loss percentage of protein, lipid and carbohydrate contents increased to 48.8%, 71.73% and 45.19% on 15th day. Preservation for a month and half resulted in 77.74%, 88.74% and 68.63% loss in protein, lipid and carbohydrate contents respectively. However, loss in protein, lipid and carbohydrate contents at –4°C were only 8.22%, 29.74% and 14.13% respectively after 7 days; 13.94%, 37.33% and 20.96% respectively after 15 days and 28.97%, 52.51% and 34.01% respectively after 45 days. Preservation at –20°C caused only negligible losses in protein and carbohydrate contents up to 7 days that were increased to only 16.17% and 17.55% respectively after 45 days. However, preservation at –20°C caused 39.96% losses in lipid contents after 45 days. Thus, preservation at –20°C is comparatively better as it exerts limited effect on protein and carbohydrate contents.

KEY WORDS: Preservation, Protein, Carbohydrate, Lipid, Zooplankton, *Moina*

INTRODUCTION

The production of zooplankton largely depends on weather and a continuous supply of fresh planktonic food for the fish is not assured throughout the year. The other factors which hamper regular supply of the zooplankton are its dependence on natural or induced phytoplankton blooms, impossibility of the culture of plankton at all locations and the difficulty in its transportation (Srivastava, 2000). Therefore, various procedures for increasing zooplankton abundance and suitable substitutes for living zooplankton have been and are still being sought. Preservation of live food is the reliable alternative and it has been achieved with varying degrees of success (Medgyesy and Wieser 1982; Fermin and Bolivar, 1994; Montaini et al., 1995).

Attempt to use frozen zooplankton was in practice for a long time for the rearing of those species of fish that do not accept artificial food (Einsele, 1949). However these attempts have not been very promising in the past (Fluchter, 1980). Kentouri (1981) reported the successful rearing of several marine species, particularly sea bass (*Dicentrarchus labrax*) with frozen plankton. Dabrowski (1984) also reported that coregonid larvae fed on live or deep frozen zooplankton showed good growth and satisfactory survival. Herring and trout assimilated more than 90% of the dry matter when fed on frozen calanoid copepod, *Calanus finmarchicus* and were healthier (Sergeant *et al*., 1979) with good survival (Dabrowski, 1984) and growth (Fermin and Bolivar, 1994). The frozen plankton float makes it easier for the fish to catch. Free amino acids are present in the frozen fluid that surrounds the zooplankton and these form a powerful attractant and appetite stimulant for fish (Tucker, 1992).

However, freezing and preservation process also results in deterioration of the nutritional values of zooplankton (Medgyesy and wieser, 1982; Srivastava, 2000). Many workers have studied the nutritional devaluation of frozen phytoplankton (Grima *et al*., 1994; Lubzens *et al*., 1995; Navarro and Sarasquete, 1998). However, little attention has been paid to evaluate the nutritional values of frozen zooplankton. The present work is an attempt to investigate effects of preservation at different temperatures (+4°C, -4°C and -20°C) and durations on the nutritional quality of cladoceran *Moina micrura* so as to find out optimum conditions of their preservation.

MATERIALS AND METHODS

***Mass culture of M. micrura*:** *M. micrura* was mass cultured in circular cemented tanks by using mixture of CD, MOC and PM (1:1:1) applied at the rate of 5.04 g/l (Srivastava and Roy, 2007). Zooplankton bloom was obtained on 18th day after inoculation.

Preservation *of M. micrura:* The plankton rich water from each culture tank was sieved through plankton net (mesh size: 53 µm) and the collected plankton was transferred to 500 ml glass beaker. After cleaning with double distilled water, the plankton was blot dried and weighed to measure the total amount of the tissue. The tissue was transferred to

cryovials to avoid the direct contact of air. These cryovials were preserved at three different temperatures- +4 ºC, –4 ºC and –20 ºC.

Biochemical estimation *of M. micrura*: The plankton samples were collected from refrigerator and deep freezer every day up to the 7th day, and then on 15th day, 30th day and 45th day at 11 am to study the effect of duration of preservation on biochemical composition of zooplankton. Fresh zooplankton samples without preservation (zero hour sample) served as control. Lowry method (Lowry *et al*., 1951), Rousch method (Rausch, 1981) and Anthrone Method (Hedge and Hofrieter, 1962) were used for estimation of protein, lipid and carbohydrate respectively. Three replicates were used for each treatment and the results were averaged. The values were expressed as mg/g of wet weight of tissue.

Statistical Analysis- Data were compared by using one-way analysis of variance (one-way ANOVA).

RESULTS

The protein content of control (fresh sample) was 202.7 mg/g wet weight of tissue (Fig. 1). Protein contents of *M. micrura* on 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 15th, 30th and 45th days of preservation at +4°C were recorded to be 197.2 mg/g, 194.4 mg/g, 190.8 mg/g, 186.5 mg/g, 183.8 mg/g, 178.3 mg/g, 169.9 mg/g, 131.2 mg/g, 105.7 mg/g and 71.4 mg/g wet weight of tissue respectively. Preservation at –4°C also resulted in decrease of protein content and it was found to be 201.8 mg/g on 1st day, 200.3 mg/g on 2nd day, 199.7 mg/g on 3rd day, 197.6 mg/g on 4th day, 194.2 mg/g on 5th day, 193.1 mg/g on 6th day, 191.5 mg/g on 7th day, 182.4 mg/g on 15th day, 168.3 mg/g on 30th day and 153.6 mg/g wet weight of tissue on 45th day of preservation. At –20ºC protein content of *M. micrura* remained unaltered up to 2nd day (48 hours) of preservation. However, there was a gradual decline in the value on 3rd, 4th, 5th, 6th, 7th, 15th, 30th and 45th days of preservation at –20°C which were recorded to be 201.7 mg/g, 201.1 mg/g, 200.3 mg/g, 198.5 mg/g, 198.1 mg/g, 192.6 mg/g, 184.5 mg/g and 176.3 mg/g wet weight of tissue respectively (Table. 1).

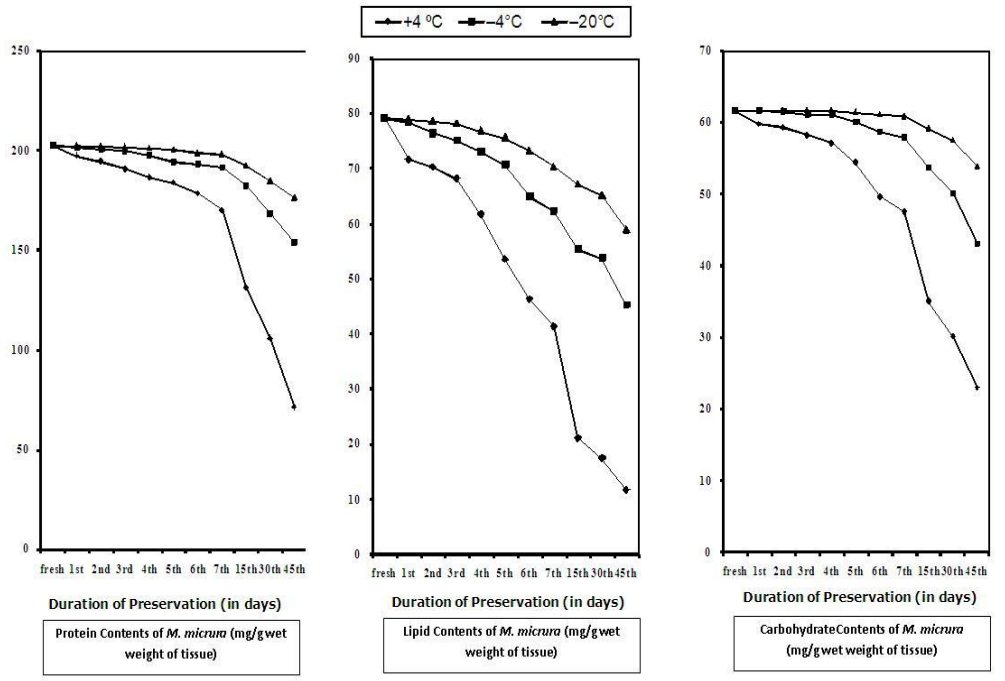

Figure: 1. Protein, Lipid and Carbohydrate contents of *Moina micrura* after various duration of preservation at +4ºC, –4ºC and –20ºC.

The fresh sample of *M. micrura* contained 79.3 mg/g lipid on wet weight basis (Fig. 1). Lipid contents of *M. micrura* on 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 15th, 30th and 45th days of preservation at +4°C were recorded to be 71.7 mg/g, 70.4 mg/g, 68.3 mg/g, 61.8 mg/g, 53.6 mg/g, 46.4 mg/g, 41.4 mg/g, 21.2 mg/g, 17.5 mg/g and 11.7 mg/g wet weight of tissue respectively. Preservation at –4ºC also resulted in decrease of lipid content and it was found to be 78.5 mg/g on 1st day, 76.6 mg/g on 2nd day, 75.3 mg/g on 3rd day, 73.2 mg/g on 4th day, 70.8 mg/g on 5th day, 65.1 mg/g on 6th day, 62.4 mg/g on 7th day, 55.5 mg/g on 15th day, 53.9 mg/g on 30th day and 45.3 mg/g wet weight of tissue on 45th day of preservation. Lipid content of *M. micrura* on 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 15th, 30th and 45th days of preservation at –20°C were recorded to be 79 mg/g, 78.7 mg/g, 78.2 mg/g, 76.9 mg/g, 75.7 mg/g, 73.3 mg/g, 70.4 mg/g, 67.2 mg/g, 65.2 mg/g and 59 mg/g wet weight of tissue respectively (Table. 1).

Carbohydrate content of *M. micrura* in fresh sample was recorded to be 61.6 mg/g wet weight of tissue (Figure 1). Carbohydrate contents of *M. micrura* on 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 15th, 30th and 45th days of preservation at +4°C were recorded to be 59.8 mg/g, 59.3 mg/g, 58.2 mg/g, 57.1 mg/g, 54.4 mg/g, 49.6 mg/g, 47.5 mg/g, 35 mg/g, 30.1 mg/g and 22.9 mg/g wet weight of tissue respectively. At –4ºC carbohydrate content of *M. micrura* remained unaltered up to 24 hours of preservation. However, there was a gradual decline in the value on 2nd, 3rd, 4th, 5th, 6th, 7th, 15th, 30th and 45th days of preservation at –20°C which recorded to be 61.5 mg/g, 61.1 mg/g, 61.1 mg/g, 60.2 mg/g, 58.7 mg/g, 58 mg/g, 53.8 mg/g, 50.1 mg/g and 43.1 mg/g wet weight of tissue respectively. At –20ºC carbohydrate content of *M. micrura* remained unaltered up to 4th day (96 hours) of preservation. However, there was a gradual decline in the value on 5th, 6th, 7th, 15th, 30th and 45th days of preservation at –20°C which recorded to be 61.4 mg/g, 61.1 mg/g, 60.9 mg/g, 59.2 mg/g, 57.5 mg/g and 53.9 mg/g wet weight of tissue respectively (Table. 1).

Table 1. Protein, Lipid and Carbohydrate Contents (in mg/g) of *Moina micrura* preserved at +4ºC, -4ºC and -20ºC
(Values given in parenthesis are percentage decrease from control value)

Day/hours	Protein			Lipid			Carbohydrate		
	+4ºC	-4ºC	-20ºC	+4ºC	-4ºC	-20ºC	+4ºC	-4ºC	-20ºC
Control (Zero hour)	202.7 ± 2.237	202.7 ± 2.237	202.7 ± 2.237	79.3 ± 1.934	79.3 ± 1.934	79.3 ± 1.934	61.7 ± 1.852	61.7 ± 1.852	61.7 ± 1.852
1st day (24 hour)	197.2 ± 2.631 (2.71%)	201.8 ± 1.082 (0.44%)	202.2 ± 2.793 (0.25%)	71.7 ± 1.15 (9.58%)	78.5 ± 2.316 (1.01%)	79 ± 3.606 (0.38%)	59.8 ± 3.557 (3.08%)	61.7 ± 0.361 (zero %)	61.7 ± 2.272 (zero %)
2nd day (48 hour)	194.4 ± 1.115 (4.09%)	200.3 ± 2.098 (1.18%)	202 ± 5.033 (0.35%)	70.4 ± 1.305 (11.22%)	76.6 ± 1.931 (3.4%)	78.7 ± 3.329 (0.76%)	59.3 ± 2.411 (3.89%)	61.5 ± 2.053 (0.32%)	61.7 ± 2.75 (zero %)
3rd day (72 hour)	190.8 ± 1.332 (5.87%)	199.7 ± 4.05 (1.48%)	201.7 ± 3.372 (0.49%)	68.3 ± 2.026 (13.87%)	75.3 ± 5.04 (3.317%)	78.2 ± 1.229 (1.39%)	58.2 ± 1.872 (5.67%)	61.1 ± 1.836 (0.97%)	61.7 ± 1.537 (zero %)
4th day (96 hour)	186.5 ± 2.117 (7.99%)	197.6 ± 3.188 (2.52%)	201.1 ± 2.352 (0.79%)	61.8 ± 1.997 (22.07%)	73.2 ± 2.691 (7.69%)	76.9 ± 2.554 (3.03%)	57.1 ± 1.93 (7.46%)	61.1 ± 0.709 (0.97%)	61.7 ± 1.267 (zero %)
5th day (120 hour)	183.8 ± 2.458 (9.32%)	194.2 ± 2.307 (4.19%)	200.3 ± 1.015 (1.18%)	53.6 ± 2.722 (32.41%)	70.8 ± 1.343 (10.72%)	75.7 ± 1.266 (4.54%)	54.4 ± 0.85 (11.83%)	60.2 ± 2.621 (2.43%)	61.4 ± 1.617 (0.49%)
6th day (144 hour)	178.3 ± 2.858 (12.04%)	193.1 ± 3.153 (4.74%)	198.5 ± 4.562 (2.07%)	46.4 ± 0.874 (41.49%)	65.1 ± 2.495 (17.91%)	73.3 ± 3.056 (7.57%)	46.6 ± 1.553 (19.61%)	58.7 ± 2.066 (4.86%)	61.1 ± 3.439 (0.97%)
7th day (168 hour)	169.9 ± 1.665 (16.18%)	191.5 ± 2.15 (5.53%)	198.1 ± 1.531 (2.27%)	41.4 ± 2.128 (47.79%)	62.4 ± 3.288 (21.31%)	70.4 ± 2.234 (11.22%)	47.5 ± 0.577 (23.01%)	58 ± 0.606 (5.99%)	60.9 ± 1.914 (1.3%)
15th day (360 hour)	131.2 ± 2.026 (35.27%)	182.4 ± 3.342 (10.01%)	192.6 ± 5.052 (4.98%)	21.2 ± 2.122 (73.27%)	55.5 ± 4.371 (30.01%)	67.2 ± 1.159 (15.26%)	35 ± 1.528 (43.27%)	53.8 ± 2.03 (12.80%)	59.2 ± 2.346 (4.05%)
30th day (720 hour)	105.7 ± 3.317 (47.85%)	168.3 ± 2.466 (16.97%)	184.5 ± 3.47 (8.98%)	17.5 ± 2.29 (77.93%)	53.9 ± 3.024 (32.03%)	65.2 ± 1.266 (17.78%)	30.1 ± 1.656 (51.26%)	50.1 ± 1.29 (18.80%)	57.5 ± 1.682 (6.81%)
45th day (1080 hour)	71.4 ± 2.629 (64.78%)	153.6 ± 1.747 (24.22%)	176.3 ± 2.572 (13.02%)*	11.7 ± 1.836 (85.25%)	45.3 ± 2.775 (42.88%)	59 ± 3.512 (25.6%)*	22.9 ± 2.371 (62.88%)	43.1 ± 1.769 (30.15%)	53.9 ± 1.477 (12.64%)*

* Significantly less than that at +4ºC at 5% level of significance.

DISCUSSION

Preservation of zooplankton at three different temperatures: +4°C, –4°C and –20°C in the present study has shown that there was decrease in nutritional quality. However, all the three parameters studied were most severely affected at +4°C but least at –20°C. Preservation at +4°C resulted in devaluation of protein, lipid and carbohydrate contents that progressively increased with progress of time. At +4°C, the loss in protein, lipid and carbohydrate contents was 16.18%, 47.79% and 23.01% after 7 day of preservation respectively. The loss percentage of protein, lipid and carbohydrate contents increased to 35.27%, 73.27% and 43.27% on 15th day. Preservation for a month and half resulted in 64.78%, 85.25% and 62.88% loss in protein, lipid and carbohydrate contents respectively. However, loss in protein, lipid and carbohydrate contents at –4°C were only 5.53%, 21.31% and 5.99% respectively after 7 days; 10.01%, 30.01% and 12.8% respectively after 15 days and 24.22%, 42.88% and 30.15% respectively after 45 days. Preservation at –20°C caused only negligible losses in protein and carbohydrate contents up to 7 days that were increased to only 13.02% and 12.64% respectively after 45 days. However, preservation at –20°C caused 25.6% losses in lipid contents after 45 days (Table. 1). Statistically, the devaluation in protein contents at –4°C was insignificantly less than that at +4°C. However, devaluation at –20°C was significantly less than that at +4°C. When the devaluation at –20°C was compared with what occurred at –4°C, the difference was insignificant. When the loss of lipid contents as a result of preservation at different temperatures was compared statistically it was found that the loss at –4°C was

alarming as it differed only insignificantly from the loss occurred at +4°C. Further, the loss of lipid contents incurred at –20°C was significantly less than what was incurred at +4°C. The loss in carbohydrate contents observed was significantly less at lower temperatures. At –20°C *M. micrura* exhibited only meager amount of losses in their carbohydrate contents, which were significantly less than that observed at +4°C. Moreover, the losses in carbohydrate contents at –4°C and –20°C did not show significant differences, suggesting that preservation at –4°C as well as –20°C both was good enough to maintain carbohydrate contents in *M. micrura*.

Thus, it is obvious from this study that preservation of zooplankton at +4°C is extremely harmful for all the three important nutritional parameters- protein, lipid and carbohydrate. However, preservation at –20°C is least harmful to protein and carbohydrate contents. A noticeable feature of zooplankton preservation at –4°C and –20°C is that there is negligible devaluation in their nutritional values up to 7th day, and after that, devaluation slightly increases. Unfortunately, even an exhaustive survey of literature provided little data on preservation of zooplankton. Therefore, for making comparison of the present observations, the available records of preservation of phytoplankton and other organisms also have been considered.

Sporadic evidences of zooplankton preservation support the present observation that while preservation at –20°C or below causes less harm to its nutritional values, preservation at higher temperature is extremely harmful. Medsey and Wieser (1982) also noted little alteration in the nutritional value of deep frozen zooplankton. Srivastava (2000) though reported remarkable decrease in protein, lipid and carbohydrate contents of *Ceriodaphnia cornuta* preserved at +4°C, short term preservation at –20°C did less harm to these values. Lubzens *et al.* (1995) reported significant reduction in concentration of certain fatty acids in *Nanochloropsis* sp. preserved at +4°C. However, preservation at –20°C caused only slight differences in its fatty acid profile. Grima *et al.*, (1994) also observed significant decrease in fatty acid contents of marine microalgae, *Isochrysis galabana* preserved at +4°C for 30 days, but at the same time, preservation at –20°C produced little effect on this value. Montaini *et al.*, (1995) has reported that freezing of *Tetraselmis susecei* at –18°C or at –196°C caused little difference in biochemical composition of biomass, like its fatty acid profile. Babarro *et al.*, (2001) have also reported significant losses in protein, carbohydrate and polyunsaturated fatty acid in frozen and freez-dried sample of the phytoplankton, *Isochrysis galabana*. Besides planktons, other crustaceans, like krill have also reported to experience significant losses in their lipid component on preservation at 251K (Kolakowska, 1986. All these reports are supporting the present observation.

However, contrary to the present observations, many workers have even reported comparatively lesser nutritional losses on preservation. But these workers have done their preservation experiments by using some chemicals as preservative or some other preservation techniques to reduce the losses in nutritional values. Goswami and Goswami (1982) preserved zooplankton sample in formalin and acetic acid. Canavate and Lubinn (1994) preserved 6 species of marine microalgae using two cryopreservation techniques- a one-step cooling technique and a two-step cooling technique with dimethyl sulphoxide, methanol and glycerol as cryoprotectant. Grima et al. (1994) also carried out preservation of marine microalgae, Isochrysis galabana, by lyophilization and freezing. However, in the present study no such preservative or preservation techniques have been used because, on one hand, the preservatives can have harmful effect on the fish that feeds on their food, on the other hand, ordinary farmers may not have access to sophisticated preservation techniques. Instead fresh samples that were simply blot dried have been used for preservation.

Deterioration in nutritional values of any preserved organic material can be easily explained in terms of enzymatic activity and bacterial action. And both of these activities are temperature dependent, i.e., higher the temperature, higher is the rate of both enzymatic activity as well as bacterial action (Kelly et al., 1978). This is why, the deterioration in the nutritional components of plankton in the present study was highest at +4°C preservation. At –4°C, enzymatic and bacterial actions are expected to be quite slow and hence preservation at this temperature causes mild alteration. A temperature as low as –20°C is expected to almost block the enzymatic and bacterial activities and hence preservation at this temperature is not expected to cause any damage to nutritional values of preserved organic materials. In the present study too, preservation at –20°C caused negligible damage to nutritional values of plankton. However, the little deterioration observed may be due to leakage of nutrients during thawing of the samples for analysis.

In conclusion, preservation of zooplankton at different temperatures can help in easy transport and steady supply of live food. The study on storage (i.e., effect of temperature and duration on biochemical composition) of *M. micrura* for 45 days at three different temperatures: +4°C, –4°C and –20°C also leads to the conclusion that preservation is suitable only at sufficiently low temperature. This information will help in the successful culture of larvae of finfish and shellfish and thus, would help in the sustainable development of aquaculture industry.

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